SEHSC®
SILICONES ENVIRONMENTAL, HEALTH AND SAFETY COUNCIL
NORTH AMERICA

Phone: 703.788.6570
Fax: 703.788.6545
www.sehsc.com
2325 Dulles Corner Boulevard
Suite 500
Herndon, VA 20171

February 20, 2008

TSCA Confidential Business Information Center (7407M)
EPA East – Room 6428
Attn: TSCA Section 8(e) Coordinator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

Contain NO CBI

Re: Supplemental TSCA Section 8(e) Submission; Preliminary Results of A 90-Day Subchronic Inhalation Toxicity Study of Methyltrimethoxysilane with a 28-Day Recovery Period in Sprague-Dawley Rats

Dear TSCA Section 8(e) Coordinator:

In accordance with the provisions of Section 8(e) of the Toxic Substances and Control Act (TSCA), as interpreted in the Statement of Interpretation and Enforcement Policy (68 Fed. Reg. 33129; June 3, 2003) and other Agency guidance, the Silicones Environmental, Health and Safety Council (SEHSC)[1], on behalf of its member companies, is providing the following information as a supplemental submission to our April 14, 2006, TSCA Section 8(e) notification, 8EHQ-0406-16442A. The initial submission reported on a 14-Day Whole-Body Inhalation Toxicity Range-Finding Study with Methyltrimethoxysilane. This supplemental submission provides preliminary results of the subsequent 90-Day Subchronic Inhalation Toxicity Study of Methyltrimethoxysilane with 28-Day Recovery Period. Neither SEHSC, nor any member company, has made a determination at this time that any significant risk of injury to human health or the environment is presented by these findings.

Chemical Substance

1185-55-3 Methyltrimethoxysilane

8EHQ-06-16442

Ongoing Study

A 90-Day Subchronic Inhalation Toxicity Study of Methyltrimethoxysilane with a 28-Day Recovery Period in Sprague-Dawley Rats – Dow Corning Corporation Study Number: 10213-102

8908000012 3

[1] SEHSC is a not-for-profit trade association whose mission is to promote the safe use of silicones through product stewardship and environmental, health and safety research. The Council is comprised of North American silicone chemical producers and importers.

310080

Summary

Whole-body inhalation exposures were conducted with Sprague-Dawley rats to average measured methyltrimethoxysilane exposure concentrations of 0, 25 ± 0.8, 99 ± 3.2, 398 ± 12.8, and 1612 ± 35.6 ppm, 5 days per week for 90 days.

There were no test article-related effects on body weight, body weight gains, food consumption, or ophthalmic findings over the course of the exposure or recovery periods for males or females. Two animals were found dead (one 400 ppm group male on study day 25; one 1600 ppm group male on study day 72). There were no abnormal clinical or gross pathological findings for the 400 ppm male. Test article-related clinical signs for the 1600 ppm male included decreased activity, soiling around muzzle, abdomen and urogenital regions with gross pathological findings including dilation of kidneys and urinary bladder along with calculus in the bladder. Test article-related clinical signs reported for all surviving animals were limited to the 400 and 1600 ppm groups and primarily included soiling of the urogenital and abdominal regions of both sexes.

Test article-related gross necropsy findings were primarily limited to the 400 and 1600 ppm group animals and included moderate dilation of the kidney and calculi in the urinary bladder. Histomorphologic changes included minimal to moderate urinary bladder hyperplasia and inflammation in all 1600 ppm group males and 9/10 females as well as 2/10 males and 3/10 females in the 400 ppm group. Kidney changes were characterized by hyperplasia of the pelvic epithelium of 4/10 males and 1/10 females, and granulomatous inflammation in 2/10 males and 1/10 females in the 1600 ppm group. The one 1600 ppm group male found dead on study day 72 demonstrated an apparent urinary obstruction possibly leading to acute uremia, with calcification of the aorta and pulmonary hemorrhagic edema as secondary effects. Additional changes included prostatic inflammation in moderate or severe degrees in 2/10 males in the 1600 ppm group. Absolute adrenal weights were increased for the 400 and 1600 ppm group females with relative weights increased at the 1600 ppm level only. This increase persisted through the 28-day recovery period. Moderate to severe inflammation of the prostate was observed in 2/10 males in the 1600 ppm group. The finding was considered to be a secondary effect resulting from urinary stasis caused by the formation of calculi leading to blockage. There were no test article-related changes in clinical pathology or serum chemistry.

Following the 28-day recovery period, calculi were observed in the urinary bladder of the 1600 ppm group males only with minimal to moderate hyperplasia of urinary bladder epithelium persisting in both males and females in the 1600 ppm group. There was no histomorphological evidence of a residual effect on the kidneys after the recovery period for the 1600 ppm group males. There were no indications of a residual effect on the prostate gland following the recovery period with control animals actually having an increased incidence of inflammation. Chronic or granulomatous inflammation of the renal pelvis, with incidence of pelvic epithelial hyperplasia, persisted in 3/10 of the 1600 ppm recovery group females.

Based on the grossly observed urinary bladder calculi and kidney dilation, along with the urinary bladder epithelial hyperplasia at the 400 ppm exposure level, the No Observable Effect Level (NOEL) for methyltrimethoxysilane vapor administered six hours per day, five days per week for a 90-day interval via whole-body inhalation exposure, to male and female Sprague-Dawley rats, was 100 ppm.

Details

Study Design

The study was conducted following the general principles of the OECD Guidelines for Testing Chemicals, "Subchronic Inhalation Toxicity 90-Day Study, No. 413, adopted May 12, 1981. Five (5) groups of 10 male and 10 female Sprague-Dawley rats were exposed to target methyltrimethoxysilane exposure concentrations of 0, 25, 100, 400, and 1600 ppm, 5 days per week for 90 days. Body weights and food consumption were evaluated weekly for each group throughout the exposure and recovery intervals. Following the exposure and recovery intervals, a gross necropsy was conducted on all animals. Several tissues were collected and processed for histological evaluation with weights collected on selected organs. Clinical pathology, hematology, serum chemistry and prothrombin times were evaluated for all animals surviving to terminal sacrifice. Upon completion of the 90-day exposure interval, an additional group of 10 males and 10 females in the 0 and 1600 ppm groups were maintained for 28-days to evaluate post-exposure recovery. Upon completion of the recovery interval, these animals were necropsied and processed identical to the exposure group animals.

Preliminary Results

Urinary Bladder:

Increased incidence and severity of epithelial hyperplasia in 10/10 males and 9/10 females at the 1600 ppm level, and 2/10 males and 3/10 females at the 400 ppm level.

Multiple calculi, ranging from mild to moderate in size, were observed in the urinary bladders of 6/10 males and 2/10 females in the 1600 ppm exposure group as well as 4/10 males in the 400 ppm exposure group.

Kidney:

Pelvic epithelial hyperplasia in 4/10 males and 1/10 females at the 1600 ppm level along with granulomatous inflammation in 2/10 males and 1/10 females at the 1600 ppm level.

Adrenal Gland:

Increased absolute weights for the 400 (18%) and 1600 ppm (25%) group females along with an increase relative to body weight (27%) for the 1600 ppm females. This finding persisted through the 28-day recovery period. In males, the 400 ppm group kidney weight was significantly increased ($p<0.05$) but a comparable effect was not seen in 1600 ppm group.

Prostate Gland:

Moderate to severe inflammation in 2/10 males, one of which died from acute urinary obstruction, at the 1600 ppm level. This is considered to be a secondary effect resulting from urinary stasis caused by urinary blockage due to calculi formation.

Actions

The SEHSC will notify EPA of any further relevant information that may be developed concerning this material. SEHSC also will provide EPA with the copy of the final report containing these study results when it is available. If you have any questions concerning this study, please contact me at (703) 788-6570, thill@sehsc.com, or at the address provided herein.

Sincerely,

Tracy Hill
Scientific Programs Manager

DOW CORNING CORPORATION
HEALTH & ENVIRONMENTAL SCIENCES
TECHNICAL REPORT

Study Title:	A 90-Day Subchronic Inhalation Toxicity Study of Methyltrimethoxysilane with a 28-Day Recovery Period in Sprague-Dawley Rats
HES Study Number:	10213-102
Test Article:	Methyltrimethoxysilane
Study Director:	Joseph M. Tobin, B.S.
Sponsor:	Silicones Environmental, Health and Safety Council 2325 Dulles Corner Blvd., Suite 500, Herndon, VA 20171
Sponsor Representative:	Wendy H. Koch, Ph.D. Epona Associates, LLC
Testing Facility:	Health and Environmental Sciences Dow Corning Corporation 2200 West Salzburg Road Auburn, MI 48611
Study Completion Date:	February 11, 2008

TABLE OF CONTENTS

TABLES

APPENDICES

1 SUMMARY

This study was conducted following the general principals of the OECD Guidelines for Testing Chemicals, "Subchronic Inhalation Toxicity 90-Day Study, No. 413, adopted May 12, 1981. The study was conducted to evaluate the toxic effects of, and subsequent recovery from, whole-body vapor inhalation exposure of methyltrimethoxysilane. Five (5) groups of 10 male and 10 female Sprague-Dawley rats were exposed to target methyltrimethoxysilane exposure concentrations of 0 (control), 25, 100, 400 and 1600 ppm, 5 days per week for 90 days. Additional satellite groups of 10 males and 10 females were included in the 0 and 1600 ppm target groups for evaluation of a 28-day post-exposure recovery period. Exposures terminated on study day 90 with non-recovery animals euthanized on study day 91. Recovery group animals remained for 28-days post exposure and were euthanized on study day 119.

All rats were exposed to vapor concentrations for six hours plus T_{99} (time required to reach 99% of the target concentration) per day, five days per week for 90 days. Average measured methyltrimethoxysilane exposure concentrations included 0, 25 ± 0.8, 99 ± 3.2, 398 ± 12.8, and 1612 ± 35.6 ppm for groups 1 through 5, respectively. Calculated nominal concentrations included 23 ± 0.4, 94 ± 1.6, 383 ± 10.4, and 1570 ± 48.9 ppm for groups 2 through 5, respectively. As a known hydrolysis product of methyltrimethoxysilane, methyl alcohol concentrations were also measured for each exposure group. Methyl alcohol was only detected at the group 5 exposure level and included a mean measured concentration of 19 ± 3.7 ppm for group 5.

Clinical observations were recorded for each animal daily with body weights and food consumption measured on a weekly basis throughout the exposure and recovery periods. Two animals were found dead (one 400 ppm group 4 male on study day 25; one 1600 ppm group 5 male on study day 72). There were no abnormal clinical or gross pathological findings for the group 4 male. Test article-related clinical signs for the group 5 male included decreased activity, soiling around muzzle, abdomen and urogenital regions with gross pathological findings including dilation of kidneys and urinary bladder with calculus in the bladder. Test article-related clinical signs reported for all surviving animals were limited to groups 4 and 5 and primarily included soiling of the urogenital and abdominal regions of both sexes.

All surviving animals were sacrificed the day following final exposure or post exposure recovery day 29, and were subjected to a full gross necropsy. Several tissues were collected and processed for histological evaluation with weights collected on selected organs. Clinical pathology, hematology, serum chemistry and prothrombin times were evaluated for all animals surviving to terminal sacrifice.

There were no statistically significant differences in overall absolute body weights or percent body weight gain in either sex across 90-day exposure group animals. There were no time of exposure differences in food consumption for either sex, in any of the 90-day exposure groups. A statistically significant decrease in absolute mean body weight was measured in group 5 recovery group females beginning exposure week four. This

significant difference persisted through completion of the exposures and into week one of the post exposure recovery period. Weekly comparison of recovery group food consumption yielded statistical differences during various weeks for males and females in both group 4 and group 5. Food consumption was similar to controls for both sexes in the group 5 recovery groups. There were no test article-related ophthalmic findings during the exposure or recovery periods.

Test article-related gross necropsy findings were primarily limited to the group 4 and 5 animals and included moderate dilation of the kidney and calculi in the urinary bladder. Histomorphologic changes included minimal to moderate urinary bladder hyperplasia and inflammation in all group 5 males and 9/10 females. Kidney changes were characterized by hyperplasia of the pelvic epithelium and/or granulomatous inflammation. The one group 5 male animal found dead on study day 72, demonstrated an apparent urinary obstruction possibly leading to acute uremia, with calcification of the aorta and pulmonary hemorrhagic edema as secondary effects. Additional changes included prostatic inflammation in moderate or severe degrees in two 1600 ppm exposure group 5 males.

Following the 28-day recovery period, calculi were observed in the urinary bladder of group 5 males only with minimal to moderate hyperplasia of urinary bladder epithelium persisting in most group 5 animals. There was no histomorphological evidence of a residual effect on the kidneys after the recovery period for the group 5 male rats. There were no indications of a residual effect on the prostate gland following the recovery period with control animals actually having an increased incidence of inflammation. Several group 5 recovery group females demonstrated chronic or granulomatous inflammation in the renal pelvis with the incidence of pelvic epithelial hyperplasia and inflammation modestly increased over controls.

In females, absolute adrenal gland weights were statistically increased in group 4 (18%) and group 5 (25%). Relative to body weight, female adrenal glands were statistically increased (27%) for group 5. There was no histological correlate and the finding was not present in males or in recovery group females. In males, group 4 kidney weight was significantly increased ($p<0.05$) but a comparable effect was not seen in group 5. After 90-days of exposure, female kidney weights relative to body weight were statistically identified as increased for group 5 (12%). This finding persisted through the recovery period.

Absolute testes and epididymide weights were statistically decreased in recovery group males exposed to 1600 ppm. This finding correlated histologically with two recovery group males showing marked testicular seminiferous tubule degeneration and corresponding epididymal oligospermia (one unilateral, one bilateral). In regular study (90-day) rats, seminiferous tubule degeneration was observed only in one control and one low-exposure (25 ppm) rats. These findings were considered common spontaneous findings in young Sprague-Dawley rats and not test article-related. There were no test article-related changes in clinical pathology or serum chemistry.

Based on the grossly observed urinary bladder calculi and kidney dilation at the 400 ppm exposure level, the No Observable Effect Level (NOEL) for methyltrimethoxysilane vapor administered six hours per day, five days per week for a 90-day interval via whole-body inhalation exposure to male and female Sprague-Dawley rats, was 100 ppm.

2 GLP COMPIANCE STATEMENT

This study was conducted in compliance with the current EPA (TSCA) Good Laboratory Practices 40 CFR Part 792. Protocol deviations are listed in Table 13. There were no deviations that adversely affected the quality or integrity of the study.

Paul A. Jean
Paul A. Jean, Ph.D.
Team Leader, Toxicology
Health and Environmental Sciences

11 Feb 08
Date

Joseph M. Tobin
Joseph M. Tobin, B.S.
Study Director
Health and Environmental Sciences

11 FEB 08
Date

3 QUALITY ASSURANCE STATEMENT

Title: A 90-Day Subchronic Inhalation Toxicity Study of Methyltrimethoxysilane with a 28-Day Recovery Period in Sprague-Dawley Rats

Study Number: 10213-102

This study has been audited by the Dow Corning Corporation Health and Environmental Sciences Quality Assurance Unit according to approved Standard Operating Procedures to assure that the raw data are accurately reflected within this final report. The following are the inspection dates and the dates inspection findings were reported.

Dates of Inspection	Phase Inspected	Findings Reported to Study Director and Management
26-30 May 06	Draft Protocol Review	30 May 06
19 Sep 06	Clinical Observations	19 Sep 06
12 Oct 06	Feeder Weight Measurements	12 Oct 06
19 Oct 06	Body Weight Measurements	19 Oct 06
08 Nov 06	Ophthalmic Examinations	10 Nov 06
14-15 Nov 06	Prothombin Time Measurements	15 Nov 06
12 Dec 06	Necropsy	15 Dec 06
14 Aug 07	Wet Specimen Verification	14 Aug 07
14-15 Nov 07	Data Book 1A including Ophthalmic Examinations, Associated Report Text and Table 12	15 Nov 07
14-16 Nov 07	Individual Scientist Reports with Histology and Necropsy Data Books	16 Nov 07
14-16 Nov 07	Draft Final Report and associated raw data related to Clinical Observations	16 Nov 07
14 Nov – 12 Dec 07	Draft Report Text & Table 13	12 Dec 07
15-16 Nov 07	Data Book 2, Inhalation Exposures (Acclimation – Day 5) including associated Tables from Appendix A	16 Nov 07
21-28 Nov 07	Data Book 4 Inhalation Exposures (Day 10-14) including tables from Appendix A	28 Nov 07
26-27 Nov 07	Data Book 3 Inhalation Exposures (Day 6-Day 9) including associated Tables from Appendix A	28 Nov 07

28 Nov 07	Data Book 5 Inhalation Exposures (Day 15-Day 18) including associated Tables from Appendix A	28 Nov 07
28-29 Nov 07	Data Books 6 and 8 Inhalation Exposures (Day 19-22 and Day 27-30) including associated Tables from Appendix A and Table 1 (Days 1-48)	29 Nov 07
29-30 Nov 07	Data Book 7 Inhalation Exposure (Day 23-26) including Tables from Appendix A/B	30 Nov 07
30 Nov 07	Data Book 9 Inhalation Exposure (Day 31-34) including associated Tables from Appendix A and Table 1 (Days 49-65)	30 Nov 07
30 Nov – 04 Dec 07	Data Book 10 Inhalation Exposure (Day 35-38) including Tables from Appendix A/B	05 Dec 07
04-05 Dec 07	Data Books 11 and 12 Inhalation Exposures (Days 39-46 including associated Tables from Appendix A and Appendix B	05 Dec 07
04-06 Dec 07	Draft Final Report and associated raw data (bodyweights and Appendix L statistics)	06 Dec 07
05 Dec 07	Data Book 14 Inhalation Exposures (Days 51-54) including associated Tables from Appendix A/B	06 Dec 07
05-06 Dec 07	Data Book 13 Inhalation Exposure (Day 47-50) including Tables from Appendix A/B	07 Dec 07
10-11 Dec 07	Food Consumption and Clinical Pathology Tables 6, 9-11, Appendices E, I, J, K, associated data books and report text	11 Dec 07
11-12 Dec 07	Individual Scientist Appendix L with Associated Raw Data Books	12 Dec 07
11-12 Dec 07	Data Book 15 Inhalation Exposure (Day 55-58) including Tables from Appendix A/B	12 Dec 07
12-13 Dec 07	Data Book 16 Inhalation Exposure (Day 59-62) including Tables from Appendix A/B	13 Dec 07
13 Dec 07	Data Book 1B	13 Dec 07
14, 17 Dec 07	Data Book 17 Inhalation Exposure (Day 63-65) including Tables from Appendix A/B	17 Dec 07

Nancy C Price — 28 Jan 08

Nancy C. Price, M.S., MPH
Dow Corning Corporation
Health & Environmental Sciences

Date

4 APPROVAL SIGNATURES

This report consists of 640 total pages, including Tables 1 – 13 and Appendices A – M.
The undersigned have read and approved this report:

Wendy A. Koch, Ph. D.
Sponsor Representative
Epona Associates, LLC

February 8, 2008
Date

Paul A. Jean, Ph.D.
Team Leader, Toxicology
Health and Environmental Sciences

11 Feb 08
Date

Joseph M. Tobin, B.S.
Study Director
Health and Environmental Sciences

11 FEB 08
Date

5 STUDY INFORMATION

Study Initiation Date:	July 13, 2006
Experimental Start Date:	August 16, 2006
Experimental Termination Date:	June 29, 2007
Study Completion Date:	February 11, 2008
Study Director:	Joseph M. Tobin, B.S.
Study Coordinator:	Christopher M. Sushynski, B.S.
Team Leader, Toxicology:	Paul A. Jean, Ph.D.
Veterinarian/Pathologist:	James W. Crissman, D.V.M., Ph.D., D.A.C.V.P. Crissman Toxicologic Pathology, LLC 2887 Oak Haven Court Midland, MI 48642
Hematology and Clinical Chemistry:	Jane M. Regan, MLT/HT (ASCP)
Statistician:	Cynthia Van Landingham, M.S. Environ Health Sciences Institute 1900 North 18th St. Suite 804 Monroe, LA 71201
Ophthalmologist:	Wendy M. Townsend, DVM, MS, Diplomat ACVO Assistant Professor of Comparative Ophthalmology Veterinary Medical Center Michigan State University East Lansing, MI 48824-1314

6 STUDY PURPOSE

The objective of this study was to evaluate the potential toxic effects of whole-body inhalation exposure of methyltrimethoxysilane for five days per week, for thirteen weeks, in male and female Sprague-Dawley rats. A sub-group of animals was maintained an additional 28-days to evaluate reversibility, persistence or delayed occurrence of potential treatment-related effects.

7 TEST GUIDELINES

This study was conducted based on the OECD 413 guideline for repeated dose inhalation toxicity testing of chemicals, adopted May 12, 1981.

8 TEST ARTICLE

The test article characterization report is presented in Appendix M. This characterization was conducted under DCC HES study number 10100-101.

Identification:	Methyltrimethoxysilane
Batch Number:	0002302681
Expiration Date:	May 2, 2008
Source:	Dow Corning Corporation, Auburn MI
CAS Number:	1185-55-3
Physical Description:	Colorless liquid
Stability:	Stable
Purity:	99.8 ± 0.3%
Solubility:	Soluble in heptane, toluene, methanol, acetone, ethanol
Storage Conditions:	Ventilated during use. All containers grounded and bonded. Kept away from heat, sparks, and moisture
Vapor Pressure:	24 mmHg at 25°C
Archive:	A reserved sample was retained

9 CARRIER

J-tube vaporization Identification:	Air

Source:	Nash® Air Compressor (Model/Size: AL-574)
Conditioning:	Passed through a series of filters to remove contaminants (Matheson® model: 460/461 and Balston® model 100-18-DX and 100-18-BX).
Chamber Make-up Identification:	Air
Source:	Building air supply
Conditioning:	Passed through a HEPA and activated charcoal filter before delivery to the chamber.

10 TEST SYSTEM

Species:	Albino Rats
Strain:	Crl: CD® (SD) IGS BR VAF/Plus®
Source:	Charles River Laboratories RTE 209 Kingston, NY 12484
Age:	Minimum of 9 weeks at experimental start
Average body weight:	Females: 203.4 – 272.4 grams at experimental start Males: 304.7 – 395.2 grams at experimental start
Sex and number used on study:	70 nulliparous and nongravid females 70 males
Number of animals ordered:	78 nulliparous and nongravid females 78 males
Number of groups:	5
Identification method Upon receipt:	Individual cage labels displaying a temporary quarantine (Q) number
Permanent identification:	IMI – 1000 Transponders (BioMedic Data Systems®) and cage labels

11 JUSTIFICATION FOR SELECTION OF TEST SYSTEM

The rat is recognized as appropriate for toxicity studies and is recommended in the OECD test guidelines. Sprague-Dawley rats (Crl: CD® (SD) IGS BR VAF/Plus®) had previously been used in a repeated-dose range finding toxicity study with this material. Ten animals/sex/group were used for each exposure levels. Groups 1 (control) and 5 (1600 ppm) contained 20 animals/sex/group each, with the additional 10/sex assigned to the 28-day recovery sub-groups.

12 METHOD OF RANDOMIZATION

Animals were ordered and acclimated to laboratory conditions to ensure adequate numbers for randomization into test groups. After release from quarantine/acclimation, animals were weight stratified by sex then randomized into groups using Provantis™. Animals were within ± 20% of the mean body weight for each sex.

13 HOUSING AND MAINTENANCE

Animal Receipt and Quarantine/Acclimation:

Upon receipt, animal resource personnel inspected each animal. Animals judged to be in good health and suitable as test animals were quarantined/acclimated for a minimum of five days. The attending veterinarian examined all animals before release from quarantine/acclimation and documented the general state of animal health.

Animal Housing

Animals were individually housed in suspended wire-mesh cages during the course of the study. The cages were elevated above Bed-O'Cobs® bedding and subjected to routine cleaning consistent with good housekeeping practices. Prior to exposure, animals were acclimated according to the following schedule:

Three days prior to exposure: Animals were placed in the exposure caging for 3.0 hours.

Two days prior to exposure: Animals were placed in the exposure caging for 6.0 hours.

One day prior to exposure: Animals were placed in the exposure caging for 6.0 – 6.1 hours in the inhalation chambers.

Following each exposure, the test article treated animals were housed in a separate animal room from the control animals.

Environmental Enrichment

Animals were given Gnaw Pucks™ and Cozee Pads™ in their home cages for environmental enrichment.

Environmental Conditions

Animals were housed in environmentally controlled animal rooms. With the exception of the deviations noted in Table 13 environmental conditions were within 18 – 26°C for temperature, 30 – 70% relative humidity, and 10 – 15 air changes per hour. Lighting was controlled to provide a 12-hour fluorescent-light/dark cycle. Temperature and humidity were recorded approximately every fifteen minutes using a HOBO® data logger (Onset Computer, Bourne, MA; software: BoxCar® Pro 4.3.1.1.). In addition, twice a day on weekdays and once a day during weekends and holidays these values were manually recorded.

Basal Diet

Certified Rodent Diet #5002, PMI® Nutritional International Inc., St. Louis, MO, was offered *ad libitum* except while animals were in the inhalation exposure cages and during the fasting period prior to terminal sacrifice. Manufacturer's periodic analyses of the certified feed for the presence of heavy metals and pesticides was reviewed by the study director to ensure that none are present in concentrations that would be expected to affect the outcome of the study.

Drinking Water

Municipal water, further purified by reverse osmosis (RO) was available *ad libitum* except while animals were in the inhalation exposure cages. Water collected from the RO system was monitored on a semi-annual basis to determine compliance with the EPA drinking water standards. The most recent analysis was reviewed by the study director to ensure that there were no contaminants known to be present in water, at levels expected to interfere with the integrity of the study.

14 ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the final rules of the Animal Welfare Act regulations (9 CFR, Parts 1, 2, and 3) and was approved by the Institutional Laboratory Animal Care and Use Committee (LACUC).

15 EXPERIMENTAL DESIGN

Route and Rationale of Test Article Administration

Route: Test article was administered by whole-body vapor inhalation.

Rationale: Whole-body vapor inhalation is an accepted method of administration for this test article. In particular, vapor inhalation exposure was appropriate as it represented a likely route of potential human exposure.

Vapor Generation

Generation of test article vapor was performed using heated stainless steel J-tubes containing stainless steel beads. Test article was metered from reservoirs into J-tubes using either a Fluid Metering Incorporated (FMI®) pump or a Harvard model syringe pump equipped with Hamilton brand glass syringes. Compressed air flowed through the J-tube at a predetermined controlled rate. The carrier/vapor mixture passed from the J-tube directed to the inlet port at the top of the exposure chamber. Just prior to entering the exposure chamber, the carrier/vapor mixture was combined with chamber supply (dilution) air where it was diluted to the target chamber concentration as it entered the exposure chamber.

Inhalation Chambers

Exposures were conducted in 2000-liter stainless steel and glass Rochester-style inhalation chambers and stainless steel exposure caging (four layers of 20 animal compartments). The animal cage position assignment within each chamber was rotated daily. Chamber temperature, relative humidity, and airflow were monitored and recorded during the acclimation period similar to that anticipated for the exposure periods. Vapor generation systems were not in operation during acclimation. The chambers were targeted for 12 – 15 chamber volume air changes per hour and environmental conditions of 20 ± 3°C and 50 ± 20%RH. Chamber airflow, temperature and relative humidity were monitored continuously and values recorded manually approximately once every 30 minutes during exposures. Chamber oxygen content was monitored, and manually recorded, once during the first day of exposure to ensure that under applied experimental conditions the oxygen content was above the minimum 19% acceptable limit.

Test Atmosphere Monitoring

The test atmosphere from each chamber was sampled by an automated sampling system. The system was designed such that test atmosphere was continuously pulled from the chamber and delivered to the analyzer through individual chamber sample lines and a stream selector valve. The sample lines were continuously purged with fresh chamber atmosphere during the entire exposure period.

Chamber atmosphere was analyzed using a gas chromatograph equipped with a flame ionization detector (GC/FID) to determine the actual chamber concentration of test article. In addition, given the potential hydrolysis of methyltrimethoxysilane in humid air, the methyl alcohol concentration within each chamber was also determined. The concentration of test article in the chamber atmosphere during the exposure period was evaluated a minimum of once every 60 minutes. As a known hydrolysis product of methyltrimethoxysilane, methyl alcohol concentrations were measured from each exposure chamber a minimum of seven times during each day's exposure period. A continuously purged sample line was used to transfer chamber atmosphere to the GC/FID for analysis.

The GC/FID methods were established prior to the experimental start date. Standard curves relating test article vapor concentration and methanol concentration to GC/FID response were established before the first exposure and then again as necessary. Preparation of calibration curves involved bag standards at five different levels that bracketed the expected range of test article or methyl alcohol chamber concentrations. The peak area attributed to the test article or methyl alcohol was plotted against the nominal bag standard concentration. Linear regression analysis of these data was performed to define the parameters of the calibration curve. Acceptance criteria for the GC/FID calibration curve included linear regression correlation coefficient of ≥ 0.98 and $\leq 10\%$ difference between the prepared bag standard concentration and the calculated bag standard concentration derived from the linear regression equation of the calibration curve.

Each calibration curve was verified prior to the exposure period by analysis of a bag standard. The bag standard actual concentration derived from the calibration curve needed to be within 10% of the bag standard nominal concentration for the calibration curve to be considered acceptable for use.

The mean daily actual measured methyltrimethoxysilane vapor concentration was compared with the daily-calculated nominal concentration as a quality control mechanism to evaluate exposure system performance. A difference of $\leq 15\%$ was considered acceptable for the 25 ppm targeted exposure level, and $\leq 10\%$ at all other target levels.

Homogeneity of test atmosphere within each chamber was evaluated once prior to initiation of animal exposures. Acceptance criteria required the mean values for each chamber zone not exceed 10% difference from the reference zone (approximate sampling location used during animal exposures).

The stability of methyltrimethoxysilane vapor in a gas bag and sample line loss was evaluated using prepared bag standards. Stability was considered acceptable over the time the measured concentration did not exceed 10% difference from the original concentration measured immediately following preparation.

Exposure chambers were leak tested prior to the first exposure to ensure proper operation. Acceptance criteria required that the mean chamber airflow measured at the inlet not be more than 10% different from the airflow measured on the exhaust side.

Exposure Levels and Treatment Regimen

Test article was administered by whole-body vapor inhalation. Animals were positioned in the chambers and the exposure period was defined as a 6-hr/day exposure at the target concentration. Animals were removed from the chambers after a second T_{99} had expired. The T_{99} period was considered as the time required for the chamber to reach 99% of the target concentration or clearance of 99% of the achieved concentration following generation of test article. Based on chamber flow rate and size, the T_{99} period was

calculated to be 23 minutes for all chambers. For practical purposes, the $T_{99} \pm 2$ minutes was used for this study.

The target exposure levels were: 0, 25, 100, 400 and 1600 ppm methyltrimethoxysilane in air. These exposure levels were determined based on results from a previously conducted 14-day range finding study (Tobin, 2007). Exposures were conducted at approximately the same time each day, five days per week over the course of thirteen weeks.

Organization of Test Groups and Exposure Levels

Group	Number of Animals	Target Exposure Concentration (ppm)
1	10 Males 10 Females 10 Males (Recovery) 10 Females (Recovery)	0
2	10 Males 10 Females	25
3	10 Males 10 Females	100
4	10 Males 10 Females	400
5	10 Males 10 Females 10 Males (Recovery) 10 Females (Recovery)	1600

Method of Euthanasia/Terminal Procedure

Scheduled Animal Euthanasia:

All surviving animals were euthanized at their scheduled termination time by exsanguination of major abdominal vessels following Isoflurane anesthesia.

Unscheduled Animal Death:

Measures were taken to minimize discomfort and pain for all animals. When the health of an animal was deemed to be unacceptable for continuation on study, the animal was euthanized by CO_2 inhalation.

Test System Observations

Mortality/Morbidity/Moribundity:

All animals were observed in their cages for mortality, morbidity, and moribundity at least twice daily on weekdays, and once daily during weekends and holidays.

Clinical Observations:

General clinical observations were made at least once a day, beginning on the first day of exposure, at approximately the same time each day. The health condition of the animals was recorded. Clinical observations included, but were not limited to, changes in the skin, fur, eyes, and mucous membranes, respiratory system, circulatory system, autonomic and central nervous systems, motor activity, and behavior patterns. General clinical observations were performed on all animals on the day of, and prior to, their scheduled necropsy.

Parameters Measured

Individual Body Weights:
Individual body weights were recorded for randomization, weekly throughout the duration of the study, then again prior to sacrifice on the day of scheduled termination.

Individual Food Consumption:
Feeder weights were recorded weekly throughout the duration of the study.

Ophthalmic Examinations:
Examinations were performed on all animals prior to group assignment. Animals with findings noted during this initial examination were not used on study. Additional examinations were conducted during the final week of exposure prior to 90-day terminal sacrifice, and during the final week of the 28-day post exposure recovery period. An indirect ophthalmoscope (following dilation using mydriatic eye drops) was used for all examinations.

Clinical Pathology:
Food was removed from all animals at least 12 hours prior to scheduled termination. Clinical pathology assessments were made on all surviving animals from which adequate samples were collected.

Blood samples were collected for hematological and clinical chemistry evaluations from all animals on the day of scheduled euthanasia as a terminal procedure. While under Isoflurane® anesthesia, a syringe and needle were used to collect blood samples from the abdominal vena cava and distributed to three sample collection tubes containing either sodium citrate, EDTA, or no anticoagulant.

Hematology:
Blood samples for the following hematology tests were collected into test tubes containing EDTA. Hematology samples were analyzed within 24 hours of collection. Analysis was performed using the Cell-Dyn 3700™ (Abbott Diagnostics, Dallas, TX).

Erythrocyte count	Hemoglobin
Erythrocyte Indices (MCV, MCH, MCHC)	Hematocrit
Leukocyte counts (total and differential)	Platelet count

Blood samples for coagulation assessment (prothrombin time) were placed into test tubes containing sodium citrate, centrifuged within two hours of collection and the plasma separated for testing. Samples were maintained at room temperature (18-24°C) or refrigerated (2-8 °C) prior to analysis. Analysis was conducted within 24 hours of collection. Analysis was preformed using the ACL 100™ (Beckman Coulter, Fullerton, CA).

Serum Chemistry:
The following serum chemistries were determined:

Alanine aminotransferase	Glucose
Albumin	Phosphate
Alkaline phosphate	Potassium
Aspartate aminotransferase	Sodium
Calcium	Total bilirubin
Cholesterol	Total protein
Chloride	Urea nitrogen
Creatinine	

Blood samples were placed into test tubes without anticoagulant, allowed to clot and centrifuged within two hours of collection and the serum separated for testing. For bilirubin and total protein analysis, the serum samples were frozen at ≤ -20°C, until analysis. Samples were analyzed using the Cobas Integra 400 plus™ (Roche Diagnostics, Indianapolis, IN).

Gross Pathology:
All animals were subjected to a full gross necropsy which included examination of the external body surface, all orifices, as well as the cranial, thoracic and abdominal cavities including contents. The following table indicates the tissues collected, weighed and processed for histopathological examination.

Organs Collected for Histomorphological Evaluation	**Organ Weights**
Adrenal	X
Heart	
Kidneys	X
Liver	X
Lung (removed intact, weighed and preserved by inflation with fixative and then immersion)	X
Spleen	
Testes	X
Ovaries	X
Naso-pharyngeal tissue (entire skull collected)	
Brain including sections of medulla/pons, and cerebral cortex	X

Eye (with optic nerve)	
Pituitary	
Thyroid/parathyroid	
Trachea	
Thymus	
Aorta	
Salivary Glands	
Spinal cord (cervical, thoracic and lumbar)	
Pancreas	
Uterus	
Esophagus	
Stomach	
Duodenum	
Jejunum	
Ileum	
Cecum	
Colon	
Rectum	
Urinary bladder	
Lymph nodes (mediastinal and mesenteric)	
Peripheral nerve	
Sternum with bone marrow	
Epididymides	X
Seminal vesicles	X
Prostate	X

Histomorphology:
Eyes were preserved in Davidson's solution until processing. Testes and epididymides were preserved in Bouin's solution for 24 – 36 hours then transferred into 70% ethanol until processing. All other tissues were placed in 10% Neutral Buffered Formalin for preservation. Tissue processing procedures included embedding in paraffin, sectioning and staining with hematoxylin and eosin for evaluation.

Histomorphological examination was conducted on all tissues collected from both the recovery and non-recovery animals in groups 1 and 5. Following initial review, additional selected tissues including urinary bladder, kidney, liver, lung and prostate were evaluated for groups 2, 3, and 4.

16 DATA ANALYSIS

Daily mean inhalation exposure concentrations and environmental conditions, along with standard deviations, were calculated using Microsoft Office Excel. Mean and standard deviation were calculated for body weights, changes in body weights, food consumption, organ weights, hematology and clinical chemistry values, using Provantis™, version 6.5.

Environmental conditions of animal rooms were monitored and recorded using a HOBO® data logger (Onset Computer, Bourne, MA; software: BoxCar® Pro 4.3.1.1.)

All data analysis was carried out using SAS version 9.1.3. Statistically significant probabilities were reported for p-values of < 0.05, <0.02, and < 0.01.

Data from the recovery groups was analyzed separately from the 90-day groups. Body weight, changes in body weight, food consumption data, organ weight, organ-to-body weight ratios, hematology data, clinical chemistry and prothrombin times were analyzed using a one-way Analysis of Variance (ANOVA) if the data satisfied the requirements of normality of the residuals and homogeneity of variance as determined using the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. If the data did not satisfy the parametric requirements, a Kruskal-Wallis test was used. If the ANOVA or Kruskal-Wallis test was significant, pair-wise comparisons of the exposed groups to control were made using the Dunnett's Test or a Wilcoxon test, respectively.

For variables with multiple measurements across time (body weight and food consumption), a repeated measurements ANOVA was performed to determine if there was a time-by-exposure group interaction.

17 RESULTS AND DISCUSSION

Exposure System and Conditions during Exposure

Results of the pre-exposure set-up indicated all chambers and analytical monitoring equipment were suitable for use with regard to chamber leak testing, homogeneity, gas bag stability, sample line loss, and GC calibration. Comparison of inlet to outlet air flow rates indicated percent differences ranging from 3 – 7% for each chamber. These results met acceptance criteria with regard to chamber leak testing. Homogeneity evaluations showed vapor concentrations of methyltrimethoxysilane were homogeneously distributed within each exposure chamber with spatial differences compared to a reference location ranging from 0 – 3.7%, 0 – 2.0%, 0.2 – 1.1% and 0 – 0.4% for groups 2, 3, 4 and 5, respectively.

Exposures were conducted in 2000-liter stainless steel and glass Rochester-style inhalation exposure chambers. Animal positioning was rotated on a daily basis in an effort to minimize potential differences. Environmental conditions were recorded approximately every 30 minutes for each chamber during all exposure periods.

The following table summarizes these conditions:

Group	Airflow (LPM)	Temperature (°C)	Humidity (%RH)
1	406 ± 2.6	24.6 ± 0.2	53.8 ± 5.7
2	404 ± 2.1	23.4 ± 0.2	52.2 ± 6.1
3	404 ± 2.6	23.6 ± 0.2	52.5 ± 6.1
4	405 ± 2.8	22.9 ± 0.2	51.2 ± 9.6
5	405 ± 2.6	24.7 ± 0.2	52.9 ± 5.7

During the first day of exposure, chamber oxygen concentrations were 20.9, 20.8, 20.9, 20.9 and 20.8% for groups 1 through 5, respectively. A summary of environmental conditions is presented in Table 1 with individual data provided in Appendix A.

Mean measured chamber concentrations of methyltrimethoxysilane were 25 ± 0.8, 99 ± 3.2, 398 ± 12.8 and 1612 ± 35.6 ppm for the 25, 100, 400 and 1600 ppm targeted exposure groups, respectively. Mean calculated nominal concentrations were 23 ± 0.4, 94 ± 1.6, 383 ± 10.4 and 1570 ± 48.9 ppm for groups 2 through 5, respectively. All daily mean measured vs. nominally calculated chamber concentration ratios were within specified acceptance limits. The mean measured methyl alcohol concentrations during the exposure intervals were below limit of calibration for groups 2, 3 and 4 and 19 ± 3.7 ppm for group 5. A summary of all chamber concentrations is presented in Table 2 with individual data provided in Appendix B.

Mortality, Morbidity and Moribundity

A summary of animal euthanasia is presented in Table 3.

Animals were observed daily for signs of mortality, morbidity and moribundity. During the conduct of the study, one male each from group 4 and group 5 was found dead and one control male was euthanized moribund. This moribund animal was euthanized due to effects resulting from maloccluded incisors. The results from this animal were not used in any statistical analysis. All other animals survived until their scheduled termination.

Body Weights

A summary of weekly mean body weights for all animals is presented in Table 4 with mean body weight gain summaries presented in Table 5. Individual animal body weight data are given in Appendix C with individual animal body weight gain data presented in Appendix D.

Mean body weights trended lower than controls over the course of the 90-day exposure period for group 5 males (~6%) and groups 4 and 5 females (~3%). There were no

statistically significant differences in overall or percent body weight gain in either sex across groups.

A statistically significant difference was seen in body weight gains across all groups in the 90-day females during weeks 1 and 2. These statistically significant differences were due to larger gains in the 100 ppm group 2 than in the other groups with none of the other groups having significant changes in body weight gains compared to the control group. There were no significant differences in the overall weight gain among the 90 day female groups.

A statistically significant difference was seen in body weight gains across all groups in the 90-day males during week 9 and 10. During week 9, the control animals gained less weight than any treated groups with statistically significant differences for the 25 ppm group 2, 100 ppm group 3 and 400 ppm group 4 males. During week 10, the control group gained significantly more than the 25 ppm and 1600 ppm groups. There were no significant differences in the overall weight gain among the 90 day male groups.

Comparison of the recovery group animals showed a statistically significant difference in group 5 female mean absolute body weights compared to controls ($p<0.05$) beginning on exposure week 4. This difference persisted through the completion of the 90-day exposure interval and into the week 1 post exposure recovery period. Following recovery week 1, mean body weights for the group 5 females remained decreased (~5%) but not significantly different from controls. Mean body weight for the group 5 male recovery animals was decreased (~5%) but not significantly different from controls throughout the entire 90-day exposure and subsequent 28-day recovery period.

Recovery group females showed a statistically significant difference in body weight gains between the 1600 ppm group and the control for weeks 4, 6, 14, 15, 16 and 18. The control groups gained significantly more weight in weeks 4, 6 and 14, significantly less in weeks 15 and 16 and lost significantly more weight in week 18 than did the 1600 ppm group. For the recovery group males, weeks 3, 7, 10 and 17 showed significant differences in body weight gains with the control group gaining significantly more than the 1600 ppm group in weeks 3, 7 and 10 and the 1600 ppm group gaining significantly more in week 17. There was no difference in the overall weight gain for either sex of the recovery group.

Food Consumption

A summary of weekly mean food consumption data for all groups is presented in Table 6. Individual animal food consumption values are given in Appendix E.

Food consumption for female 90-day exposure animals was increased for groups 3 ($p<0.02$) and group 4 ($p<0.05$) compared to control during week 13. For males, group 3 showed a significant increase ($p<0.02$) in food consumption compared to controls during week 9. Food consumption was similar to controls for both sexes in each group during

all other weeks. There were no time of exposure differences in food consumption for either sex, in any of the 90-day exposure groups.

Food consumption in the recovery group animals was significantly decreased ($p<0.05$ or 0.02) during weeks 4 and 5 for the group 5 females and weeks 6, 7, 8 and 10 for the group 5 males compared to controls. Food consumption was similar to control for both sexes in group 5 during each week of the 28-day post exposure period.

Clinical Observations

A summary of daily clinical observation for all groups is presented in Table 7, with individual clinical observations presented in Appendix F.

One control group male was euthanized moribund prior to scheduled terminal sacrifice. Clinical signs for this animal included a missing tooth, various staining, hair standing up, and ungroomed appearance. This animal was euthanized following repeated loss of body weight and reduced food consumption during weeks 8 and 9 which were considered a result of the animal's inability to properly eat. One male each from group 4 and group 5 was found dead during the course of the study. There were no abnormal clinical signs up to the time the animal was found dead for the group 4 male. Clinical signs for the group 5 male included decreased activity, maloccluded incisors and soiling around the muzzle, abdomen, urogenital regions, limbs and paws. Clinical signs were evaluated daily until scheduled termination for all other surviving animals.

Clinical signs across all groups included excessive hair loss and soiling in various animals of each sex. Both sexes of groups 4 and 5 included general soiling as well as a more frequent incidence of excessive soiling around urogenital and abdominal regions seen immediately following exposure. One palpable mass was reported in a group 3 male. The mass was confirmed at the time of necropsy as being the microchip transponder.

Gross Macroscopic Evaluations

Individual animal macroscopic findings are presented in Appendix G

Notable macroscopic observations included urinary bladder calculi in four (4) males and one (1) female in group 5 and two (2) males in group 4. In addition, one (1) group 5 male also exhibited dilation of both the urinary bladder and kidneys. In separate group 5 males, one contained red, discolored mediastinal lymph nodes and two (2) in the recovery group with a decrease in size of testes. One (1) group 3 female exhibited an ovarian mass approximately 1.1 cm – 3.0 cm in size. There were no other significant macroscopic observations noted.

Organ Weights

A summary of organ weights along with organ to body weight ratios is presented in Table 8, with individual weights and ratios presented in Appendix H.

Absolute adrenal weights were significantly increased compared to controls for group 4 and 5 females. Adrenal and kidney-to-body weight ratios were also significantly increased compared to controls for group 5 females; a significant increase in kidney-to-body weight ratio remained in the group 5 recovery group females. These findings are considered exposure-related but histological evaluation suggests there is no apparent alteration in tissue. These effects were not present in the recovery group animals and therefore not considered adverse. There were no other significant organ weight differences for females.

In males, absolute kidney weights were significantly increased compared to controls for group 4 but not group 5. In addition, there was a significant decrease in absolute testes and epididymides weights for the group 5 recovery males. This finding correlated histologically with two recovery group males having marked testicular seminiferous tubule degeneration and corresponding epididymal oligospermia (one unilateral, one bilateral). In the 90-day exposure males, seminiferous tubule degeneration was observed in one control and one group 2 rats. Given these are common spontaneous findings in young Sprague-Dawley rats; it is unclear if these findings are test article-related. There were no significant differences observed in any organ to body weight ratios for the males.

Histopathology

Summaries and individual histomorphological results are provided in Appendix G.

Following histological examination, the urinary bladder and kidneys were identified as primary target organs with possible secondary effects in the prostate.

There was minimal to moderate urinary bladder hyperplasia and inflammation in all males and 9 of 10 females in group 5. Urinary bladder calculi were observed in both sexes of group 5 and were distinct from the calculi observed in the lower exposure level male groups. In the group 5 animals, the exposure-related calculi were fragmented in tissue processing, but the remaining portions were non-staining, gray-appearing, crystalline matter that often had a lamellar appearance. In male rats, this was often associated with the normal proteinacious plugs. In those cases, the finding was recorded simply as "calculus" to distinguish it from the pure proteinacious plugs.

Kidney changes were much less consistent; more varied than urinary bladder changes, and were most often characterized by hyperplasia of the pelvic epithelium and/or granulomatous inflammation. The latter change, though infrequent, could be rationalized to be a foreign body type reaction to trapped amorphous crystalline material, a change not encountered in control animals. The trapped material was similar in appearance to the urinary bladder calculus material.

The severity of the urinary tract changes depended partially on whether there was some degree of urinary obstruction, although, since calculi were observed much less often than hyperplasia, it may be that crystaluria was enough to cause hyperplasia of the epithelium lining the renal pelvis and urinary bladder. One group 5 animal found dead exhibited urinary obstruction and acute uremia, with calcification of the aorta and pulmonary hemorrhagic edema as secondary effects.

Prostatic inflammation, which in minimal or mild degrees is fairly common, occurred in moderate or severe degrees in two group 5 males, one of which was found dead.

Following the 28-day recovery period, urinary bladder calculi were observed in all of the group 5 males. Minimal to moderate hyperplasia of urinary bladder epithelium persisted in most rats. Chronic or granulomatous inflammation in the renal pelvis was observed in several female rats.

In male rats, there was no histopathological evidence of a residual effect on the kidneys after the recovery period. In females, the incidence of pelvic epithelial hyperplasia and inflammation was modestly increased over controls. In one female, the inflammation was described as granulomatous.

There were no indications of a residual effect on the prostate gland following the recovery period. No animals had more than mild inflammation of the prostate gland, and the incidence of inflammation was higher in control animals.

No additional exposure-related findings were detected in other examined tissues. The remaining findings generally occurred infrequently or were common spontaneous findings in rats of this age and strain.

Clinical Chemistry

A summary of clinical chemistry results is presented in Table 9 with individual data provided in Appendix I.

Comparison of the 90-day exposure females to controls showed slight differences ($p<0.01$) in both total bilirubin and alkaline phosphate in group 5, and alkaline phosphate ($p<0.02$) in group 2 with all values decreased compared to controls. In males, the group 4 creatinine levels were significantly decreased from controls ($p<0.05$). These statistically significant findings were spurious, not exposure-related, and not in the direction of pathological change. All other parameters evaluated were considered similar to controls.

There were no significant differences noted in males or females in the 28-day recovery group.

Hematology

A summary of hematology results is presented in Table 10 with the individual data presented in Appendix J.

There were no statistically significant differences in hematology results or changes to hematology parameters attributable to test article exposure for either sex of the 90-day exposure or 28-day recovery groups.

Prothrombin time

A summary of prothrombin times is presented in Table 11 with the individual data presented in Appendix K.

There were no statistically significant differences in prothrombin time attributable to test article exposure for either sex of the 90-day exposure or 28-day recovery groups.

Ophthalmic examinations

A summary of ophthalmic examinations is provided in Table 12. Observations following exposure included chorioretinal hypoplasia in one (1) group 2 male, poor dilation in one (1) group 3 female, and a miotic pupil in one (1) group 4 female. There were no abnormal ophthalmic findings noted following the recovery period. The spontaneous findings noted were considered not to be attributed to test article exposure.

Statistical analysis

A summary of all statistical analysis is presented in Appendix L

18 CONCLUSIONS

Administration of methyltrimethoxysilane at target vapor concentrations of 0, 25, 100, 400 and 1600 ppm to male and female Sprague-Dawley rats via whole-body inhalation for six hours per day, five days per week, for 13 weeks resulted in mortality of one group 4 and one group 5 male.

Test article-related clinical signs were limited to the 400 ppm and 1600 ppm exposure groups and included increased incidence of soiling around urogenital and abdominal regions. Although not statistically significant, body weights for the group 5 males and 4 and 5 females trended lower than controls over the entire exposure interval (5-6%). This trend was characterized by an initial decrease in body weight gain during the second week of exposure with weight gains generally comparable to control throughout the remaining exposure and recovery intervals.

Test article-related observations following inhalation exposure to methyltrimethoxysilane were primarily attributable to its effects on the urinary tract. There were no clinical chemistry or hematological effects attributable to test article exposure. Organ weights were minimally impacted, with a small, possible stress-related increase in adrenal gland weights in the 90-day, 1600 ppm group 5 females, and a small increase in kidney weight in 90-day and recovery group 5 females. At 1600 ppm, test article exposures often led to formation of grossly observed urinary calculi. These could be observed grossly as large single or small granular calculi in the urinary bladder. Microscopically, these calculi

were observed at the center of foci of granulomatous, inflammation in the renal pelvis, as fragments of crystalline material in the urinary bladder, or incorporated into the proteinacious calculi (plugs) common in male rats. In most group 5 animals (1600 ppm), and a few group 4 animals (400 ppm), there was some degree of diffuse urinary bladder epithelial hyperplasia as well as urinary calculi which persisted through the recovery period. There was no indication of this in the 100 ppm group 3 exposure group.

A statistically significant decrease in absolute testes and epididymides weights was observed for the group 5 (1600 ppm) recovery males. This finding correlated histologically with marked testicular seminiferous tubule degeneration and corresponding epididymal oligospermia. In the 90-day exposure males, seminiferous tubule degeneration was observed in one control and one group 2 rat. Given these are common spontaneous findings in young Sprague-Dawley rats; these findings are not believed to be test article-related. There were no significant differences observed in any organ-to-body-weight ratios for the males.

Test article-related effects in 400 and 1600 ppm exposure group included increased incidence of urogenital and abdominal soiling, mild inhibition of body weight gain early in the treatment period leading to reduced absolute weights throughout the 90-day exposure interval for the 400 ppm group 4 females and the 1600 ppm group 5 males and females with recovery of the group 4 females following cessation of exposures. Formation of calculi in the urinary bladder is the leading cause of secondary gross and histological effects seen following exposure to methyltrimethoxysilane. The composition of these calculi and the mechanism leading to their formation could not be confirmed based on the results of this study. Calculi formation may be dependent on reaching a threshold concentration of either methyltrimethoxysilane, or its subsequent metabolites, sufficient enough to trigger precipitation.

Based on the clinical signs along, grossly observed urinary bladder calculi, and kidney dilation at the 400 ppm exposure level, the No Observable Effect Level (NOEL) for methyltrimethoxysilane vapor administered six hours per day, five days per week for a 90-day interval via whole-body inhalation exposure to male and female Sprague-Dawley rats, was 100 ppm.

19 ARCHIVE

All work product including the protocol, amendments and deviations (if applicable), study authorization form, raw data, correspondence, samples/specimens, and final report will be retained for up to five years at the HES Archives, Dow Corning Corporation, 2200 West Salzburg Road, Auburn, MI 48611. Thereafter, the Sponsor will be contacted for the disposition of the archived material. The Sponsor will then assume responsibility for the archived materials and any expense for further archiving, disposal, and/or shipment.

20 REFERENCES

Kallos, G.J., Tou, J.C., Malczewski, Regina M., Boley, William F., Stability of Alkoxysilanes in Humid Air and Aqueous Media, Report Number: 1988-I0039-20

Organization for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals. Section 4, No. 413: Subchronic Inhalation Toxicity: 90-day Study. Adopted May 12, 1981.

Stutts DH, Characterization of Methyltrimethoxysilane, CAS# 1185-55-3 (Batch# 0002302681), TIS Report Number: 2005-I0000-55422.

Tobin, J.M., A 14-Day Whole Body Inhalation Toxicity Range Finding Study of Methyltrimethoxysilane in Rats, DCC Study Number: 10079-102, TIS Report Number: 2007-I0000-58134.

U.S. Department of Agriculture, Animal Welfare Act, 9 CFR Subchapter A, Parts 1, 2 and 3.

U.S. Environmental Protection Agency, Good Laboratory Practice Standards, 40 CFR Part 792.